# Expression, action and function of phosphoinositide 3-kinase p110delta

Kok, Klaartje

# Chapter 1

# Introduction,
# Aim and
# Outline of this thesis

Klaartje Kok

**Phosphoinositides and Phosphoinositide 3-Kinases**

Phosphoinositides (PIs) are phospholipid components of eukaryotic cell membranes. By functioning as second messengers they play an important role in signal transduction. Phosphatidylinositol (PtdIns) is the building block of phosphoinositides and consists of a hydrophobic tail, composed of two fatty acids chains, that is anchored into the lipid bilayer, connected to a hydrophilic inositol head group that resides in the cytoplasm. The inositol ring has free hydroxyl groups that can be phosphorylated in different combinations. The term phosphoinositide (PI) applies to any phosphorylated derivate of phosphatidylinositol [1,2] (Figure 1).

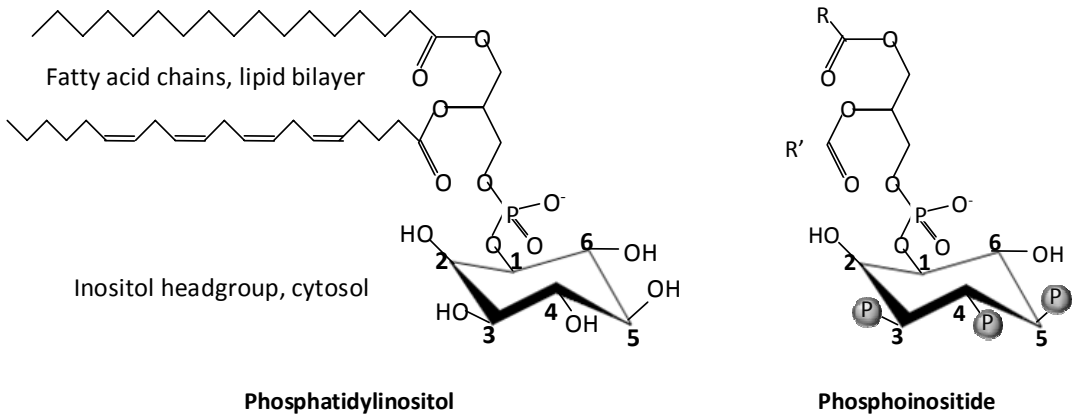


**Figure 1: Simplified chemical structures of phosphatidylinositol (PtdIns) and phosphoinositide (PI).** PtdIns consists of a cytoplasmic inositol headgroup and a hydrophobic tail consisting of two fatty acid chains that are incorporated into the inner leaflet of the membrane lipid bilayer. (Adapted from Nock [3])

Eight PI species have been indentified in eukaryotic cells. They are produced as a result of the combined action of various kinases, phosphatases and phospholipases [1] (Figure 2). Phosphatidylinositol is the most abundant PI in mammalian cells, with basal levels up to 20 times higher than those of PtdIns(4)P and PtdIns(4,5)P2, the most abundant mono- and disphosphorylated PIs, respectively. The levels of PtdIns(3,4,5)P3 vary, but are comparable to those of PtdIns(3,4)P2 and PtdIns(3,5)P2 which together constitute less than 1% of the disphosphorylated PIs. That such little amount of the total inositol-containing lipids are phosphorylated at the 3-position, is consistent with the idea that these lipids exert specific regulatory functions inside the cell, as opposed to a structural function [1,4].

Four species of 3'-PIs have been indentified in eukaryotic cells, PtdIns(3)P, PtdIns(3,4)P2, PtdIns(3,5)P2 and PtdIns(3,4,5)P3. With the exception of PtdIns(3,5)P2, these 3'-PI species are generated by the action of phosphoinositide 3-kinases (PI3K).

Upon cellular stimulation the levels of PtdIns(3,4)P2 and PtdIns(3,4,5)P3 rise sharply. Class I PI3Ks are responsible for the generation of PtdIns(3,4,5)P3 (see below) [1,5].

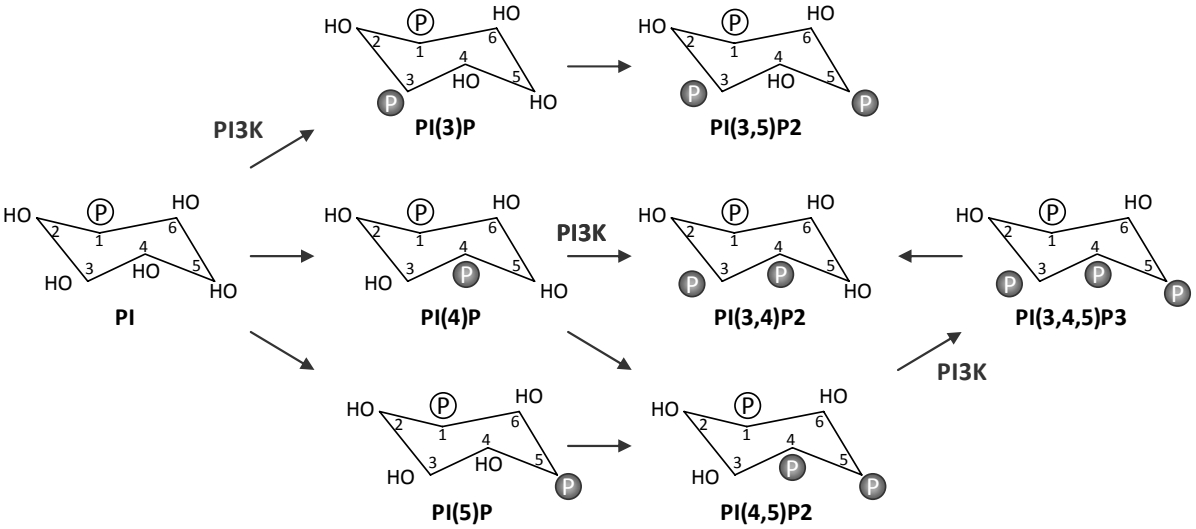


**Figure 2: Schematic representation of the eight eukaryotic phosphoinositide (PI) species**. The eight PI species are produced as a result of the combined action of various kinases, phosphatases and phospholipases. Here, the PI species and phosphoinositide 3-kinases (PI3K) are shown. (Adapted from Nock [3])

## PI3K

PI3Ks catalyse the transfer of the γ-phosphate group of ATP to the 3'-OH position of the inositol ring of PtdIns and PI lipids, producing 3'-PI lipid derivatives [6,7]. In mammalian cells these lipid derivatives are PtdIns(3)P, PtdIns(3,4)P2 (or PIP2) and PtdIns(3,4,5)P3 (or PIP3) [1]. The 3'-PIs are second messengers that activate signalling pathways downstream of PI3K through recruitment of proteins containing pleckstrin homology (PH), FYVE (for conserved in Fab1, YOTB, Vac1, and EEA1) or Phox-homology (PX) binding domains [8-10]. At the membrane these effector proteins become activated and initiate various signalling cascades [2,9,11].

### Classification of PI3Ks

The PI3K family has been divided into three classes of enzymes (Class I, Class II and Class III), based on their structure, mode of activation and *in vitro* lipid substrate specificity [7].

## Class I PI3K

Class I PI3Ks are heterodimers consisting of a 110 kDa catalytic subunit associated with an adaptor/regulatory subunit (Figure 3). The *in vitro* substrates for class I PI3Ks are PtdIns, PtdIns(4)P or PtdIns(4,5)P2, however their preferred *in vivo* substrate appears to be Ptd(4,5)P2, which results in PIP3 production [1]. Class I PI3Ks have been further categorized into class IA and class IB, on the basis of whether they are activated downstream of tyrosine kinases or G-protein coupled receptors (GPCRs), respectively. The members of both classes also bind to Ras [12]. Detailed discussion regarding the tissue distribution of Class I PI3Ks, the genes encoding them as well the regulation of their expression in health and disease is provided in *Chapter 2.*

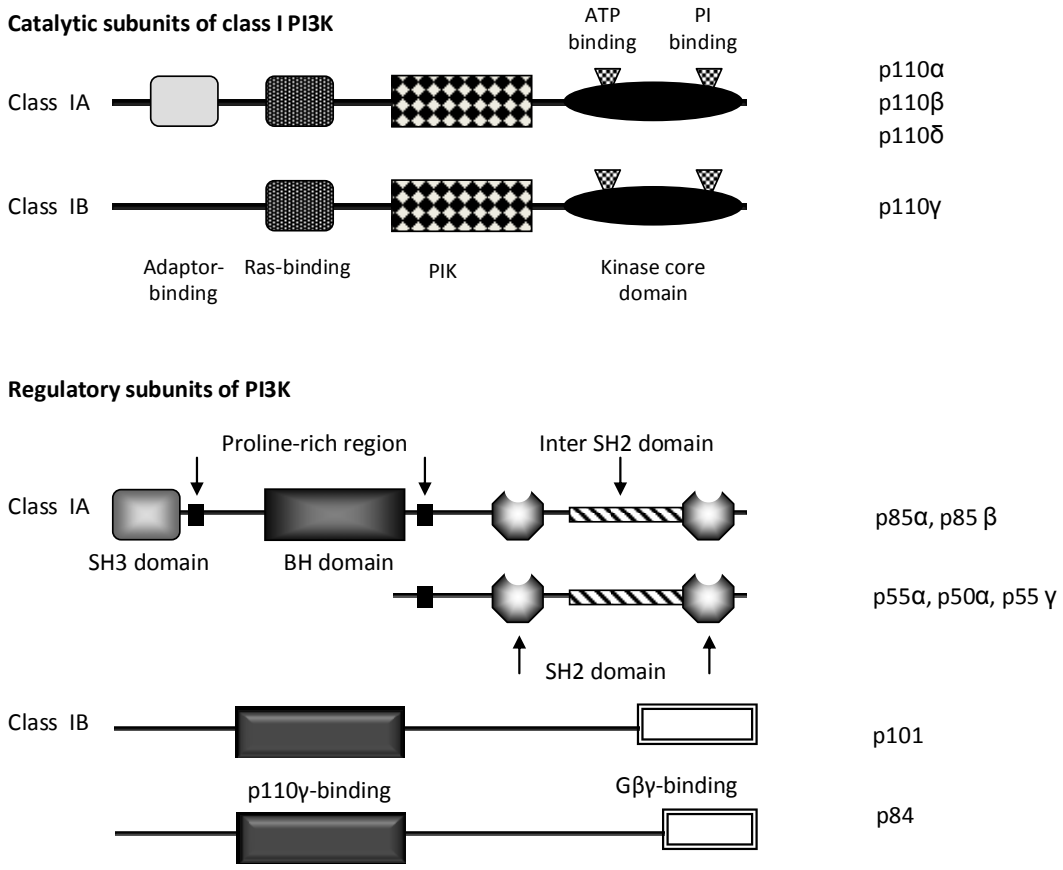


**Figure 3: Modular structure of class I PI3K catalytic and regular subunits**. The class I PI3K catalytic subunits all contain a PIK (PI kinase homology) domain and a kinase core domain, which contains both the ATP and PI binding sites. Class IA catalytic subunits (p110α, p110β and p110δ) all have a p85 adaptor binding domain. The sole class IB catalytic subunit, p110γ does not contain the p85 adaptor binding domain. At least five class IA regulatory subunits exist. p85α, p55α, p50α, p85β and p55γ. All class IA regulatory subunits have two SH2 domains and an inter SH2 domain that is essential for binding the p110 catalytic subunits. p85α and p85β also have an additional Bcr homology (BH) domain. There are two class IB regulatory subunits, p101 and p84. The domains illustrated for p101 and p84 are not yet well defined. A region at the N-terminus of p101 has been identified to be essential for binding to p110γ and a separate C-terminal region mediates p101 binding to GPCRs [13]. (Adapted from Nock [3])

**Class IA PI3Ks**

The mammalian catalytic subunits of class IA PI3Ks are p110α, p110β and p110δ [14-16]. They are encoded by three separate genes. They form heterodimers with one of five regulatory subunits, p85α, p55α, p50α, p85β and p55γ, collectively named 'p85s' [17-24]. p110α and p110β are ubiquitously expressed and knockout mice are embryonically lethal [25-27] while p110δ is highly enriched in leukocytes [16]. The PI3K regulatory subunits contain two Src homology 2 (SH2) domains to which the p110 catalytic subunit binds [2]. All of the p110 catalytic subunits are able to bind all the regulatory subunits and there is no known specificity for a particular p110 to bind a particular p85. The association of the regulatory subunit with p110 stabilizes the catalytic subunit but inhibits its catalytic activity [28].

Upon activation of protein tyrosine kinases, p110 catalytic inhibition is relieved through engagement of the regulatory subunit SH2 domains with phosphotyrosine residues in a specific Y(P)xxM motif [28]. The protein tyrosine kinases involved can be integral to the receptor itself, as is the case for many growth factor receptors (Figure 4) or can be activated by direct or indirect association to the receptor, such as the Src family of protein tyrosine kinases associated with receptors for antigen or antibodies [29]. Likewise, the critical tyrosine residues that are phosphorylated to mediate p50/p55/p85 binding, may be located on the receptor itself, such as PDGF receptor, or on receptor-associated proteins (or adaptors), such as IRS (insulin receptor substrate) [29]. Through binding Y(P)xxM motifs, the regulatory subunit serves to transport the p110 subunit to the cell membrane where it is brought into close proximity with its lipid substrate Ptd(4,5)P2 to generate PIP3 [29].

**Class IB PI3K**

This class of PI3K enzymes exists of a 110 kDa catalytic subunit, p110γ [30] that can bind to a p101 [31] or p84 [32,33] regulatory subunit. As a heterodimer, p110γ is activated by the Gβγ units of heterotrimeric G proteins [31,34]. p110γ plays an essential role in number of physiological processes such as neutrophil chemotaxis, mast cell degranulation and cardiac function.

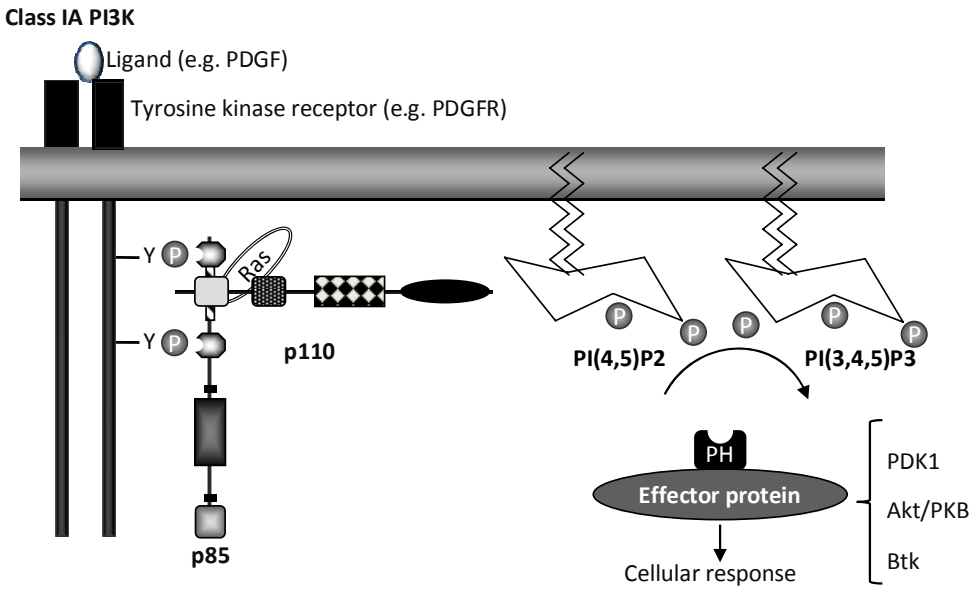


**Figure 4: Activation of class IA PI3K.** Class IA PI3Ks are activated by receptors activating protein tyrosine kinases. The schematic represents the activation of Class IA heterodimers by a growth factor receptor with intrinsic protein tyrosine kinase activity. Ligand-induced dimerisation of the receptor results in phosphorylation of multiple key tyrosine residues in the cytoplasmic tails, some of which are within YxxM motifs that act as docking sites for the SH2 domains of the class IA regulatory subunits. This leads to recruitment of the p110 catalytic subunit to the plasma membrane where it can phosphorylate PI (4,5)P2 to PIP3. GTP-bound Ras is also known to bind the catalytic subunits of class I PI3Ks. (Adapted from Nock [3])

### Class II and Class III PI3Ks

Class II and III PI3Ks preferentially phosphorylate PtdIns to produce PtdIns(3)P. Class II enzymes do not form a complex with a dedicated regulatory subunit, yet a number of ligands that modestly activate these enzymes have been identified, including insulin [35-37], epidermal growth factor [38], platelet-derived growth factor (PDGF) [38], chemokines/cytokines [39-41] and integrins [42]. The biochemical mode of class II PI3K activation is not entirely clear, but might involve recruitment via the Grb2 (growth factor receptor-bound protein 2) adaptor (as seen for PI3K-C2β [43]) or through binding to the clathrin heavy chain (documented for both PI3K-C2α and PI3K-C2β [44,45]). The importance of class II PI3Ks in cell signalling and biology, relative to that of class I PI3Ks, is not clear at the moment [46,47]. Vps34 (named for vacuolar protein sorting), the sole class III PI3K, exists as a heterodimer bound to the Vps15 regulatory subunit (also called p150 in mammals) [48]. Vps34 has been implicated in endocytosis, autophagy and nutrient signalling through the mTOR (mammalian target of rapamycin) pathway [48]. More information regarding class II and class III PI3K is given in Box 1 in *Chapter 2* of this thesis.

**Lipid Phosphatases and Phospholipases**

The level of PIs is also regulated by several phosphatases. The class I PI3K lipid products can be dephosphorylated by three main phosphatases: PTEN (phosphatase and tensin homolog deleted on chromosome ten), SHIP1 (SH2 domain-containing inositol 5-phosphatase) and SHIP2. PTEN and SHIP2 are broadly expressed while SHIP1 is expressed primarily in hematopoietic cells [49]. PTEN is a well-known tumour suppressor gene and is frequently inactivated by mutation, gene deletion, or epigenetic silencing [50,51]. PTEN has 3-phosphatase activity and regulates PI3K-dependent signalling events through dephosphorylation of PIP3 to PtdIns(4,5)P2, thereby preventing membrane-localization of PH domain-containing effectors. SHIP1 and SHIP2 are both 5'phosphatases which dephosphorylate PIP3 to PtdIns(3,4)P2 [52,53]. As mentioned above, PH domain containing proteins can interact with PIP3, however certain PH domains exhibit dual specificity for PIP3 and PtdIns(3,4)P2, such as DAPP1 (dual adaptor of phosphotyrosine and 3-phosphoinositides 1), whereas as others exhibit selectivity toward PtdIns(3,4)P2, such as TAPP (tandem PH domain-containing protein). The phosphatase activity of SHIP can therefore regulate PI3K-dependent signalling events by redirecting the signalling pathway through another set of 3'PI lipid-binding effectors [51]. In comparison to PTEN, SHIP genes are not widely thought of as tumour suppressors although there have been a few reported instances of *SHIP* mutations in a proportion of acute, myeloid and lymphoblastic leukaemias [54,55].

Finally, the levels of different PIs are regulated by phospholipases. A well-characterised signalling pathway is the PLC/PtdIns(4,5)P2 pathway which involved phospholipase C that cleaves PtdIns(4,5)P2 into membrane bound diacylglycerol and soluble Ins(1,4,5)P3 that in turn promote PKC activation and a rise in intracellular and calcium levels, respectively [56].

**Signalling Downstream of Class I PI3K**

Stimulation of class I PI3K leads to a rapid rise of the PIP3 levels at the inner leaflet of the plasma membrane. A wide variety of cell-surface receptors stimulate class I PI3Ks, such as inflammatory stimuli, growth factors, neurotransmitters, hormones and antigens [29]. The raise in PIP3 levels leads to recruitment of effector proteins that usually contain PH domains. At the membrane these effectors become activated and initiate a wide array of responses (Figure 5) [1]. The effector proteins include serine/threonine kinases, tyrosine kinases, adaptor proteins and nucleotide exchange factors [2,29]. The major effector of the class I PI3K signalling pathway, Akt (also known as PKB), will be discussed in this introduction.

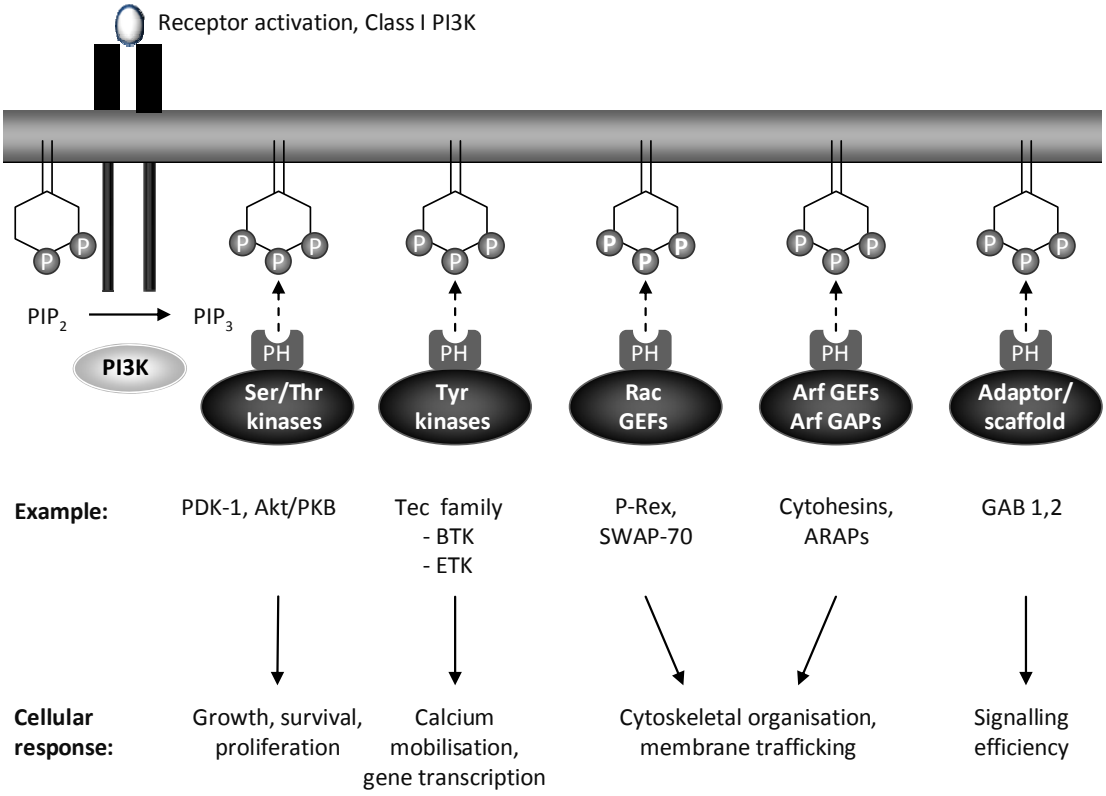


**Figure 5: Downstream effectors of class I PI3K.** There are a number of PH domain containing proteins that act as downstream effectors of class I PI3K activation. These include Ser/Thr kinases, tyrosine kinases, regulators of small GTPases such as GAPs and GEFs and adaptor or scaffold proteins. These effector proteins regulate diverse cellular responses such as cell growth, cell survival, gene transcription and cytoskeletal organisation. (Adapted from Nock [3])

Akt has a fundamental role in many cellular processes such as metabolism, cell survival, and proliferation (Figure 6) [57-59]. In inactive cells, Akt resides in the cytosol in a low-activity conformation. PI3K activation and subsequent rise in PIP3 levels result in translocation of Akt to the plasma membrane where Akt becomes phosphorylated at the Serine (Ser) 473 and Threonine (Thr) 308 residues [57,59]. Full activation of Akt requires phosphorylation of both residues [60]. In addition to translocating Akt to the plasma membrane, the interaction of the PH domain of Akt with PIP3 relieves Akt intermolecular inhibition, allowing the activated complex to dissociate and modify its targets [57]. Phosphorylation of Thr308 is carried out by another PH-domain containing protein kinase named PDK1 (3-phosphoinositide-dependent kinase-1) [61] while the rictor-mTOR complex directly phosphorylates Akt on Ser473 and facilitates Thr308 phosphorylation by PDK1 [62].

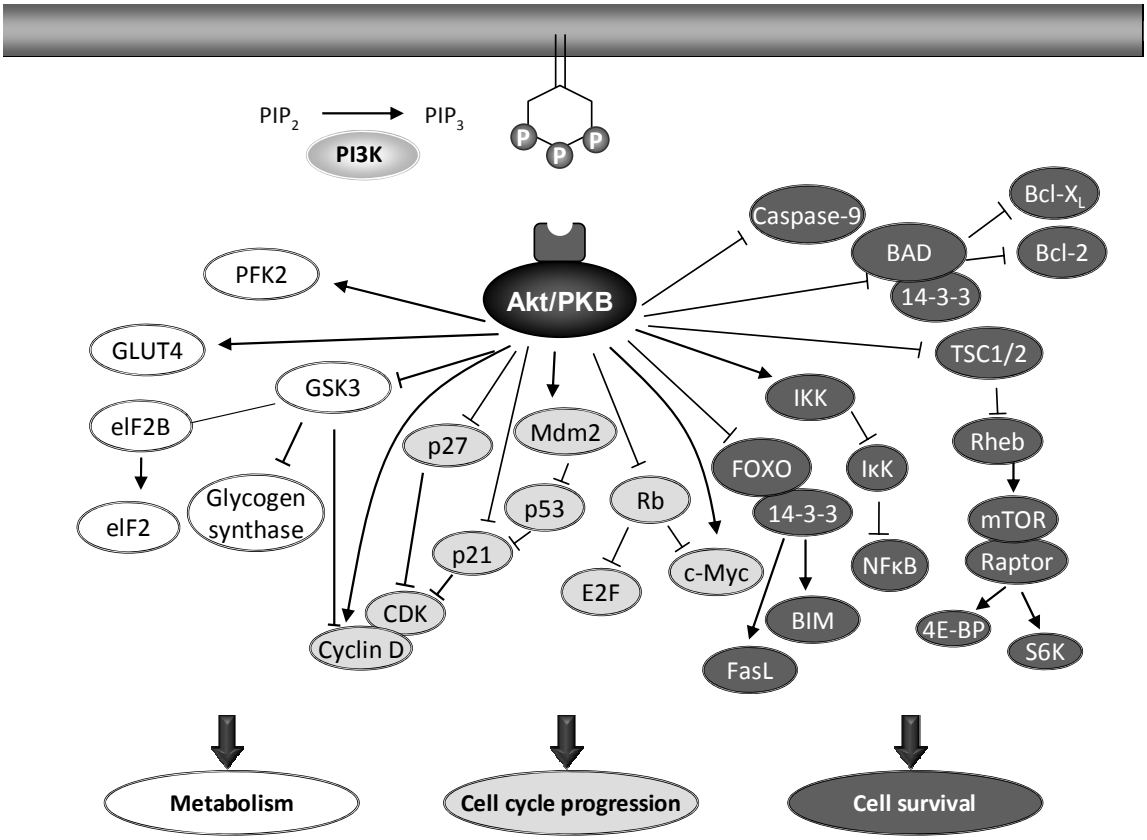


**Figure 6: Signalling downstream of Akt activation.** ***Metabolism:*** Akt activation regulates cell metabolism through phosphorylation and inhibition of GSK3 resulting in increased activation of glycogen synthase and the transcription factor eIF2B. PFK2 and GLUT4 are also activated downstream of Akt, which promote glycolysis and glucose uptake respectively. ***Cell cycle progression:*** Akt activation promotes cell cycle progression through inhibiting p27 and p21, which would otherwise inhibit CDKs. Mdm2 is phosphorylated and activated by Akt, which inhibits the tumour-suppressor activity of p53. Akt-induced inactivation of the tumour-suppressor retinoblastoma (Rb) and activation of c-Myc also promotes cell cycle progression. ***Cell survival:*** Akt promotes cell survival through inhibition of the FOXO family of transcription factors, which induce transcription of pro-apoptotic proteins FasL and BIM. The pro-apoptotic proteins Caspase-9 and BAD are also inhibited by Akt. Akt activates NF-κB-induced transcription by activating IKK which in turn phosphorylates and causes the degradation of inhibitor of NFκB, IκB. Akt phosphorylates and suppresses the TSC1/2 complex, resulting in increased activation of the Rheb GTPase and enhanced mTOR activity. The main downstream targets of mTOR are 4EBP and S6K. (Adapted from Nock [3])

**The importance of PI3Ks**

PI3Ks generate second messengers that regulate a broad variety of cellular responses such as growth, proliferation, survival, differentiation, intracellular traffic and cell migration [1]. It is therefore to be expected, that deregulation of function of PI3Ks is involved in many diseases. Indeed, PI3K are key players in many diseases such as diabetes, cancer, allergy and inflammation and are therefore considered attractive targets for therapeutic intervention. However, given the importance of PI3Ks in a multitude of signalling event in all cells, inhibition of ubiquitously expressed enzymes will be toxic for the organism. Hence it important to increase understanding of the expression, action and function of PI3Ks. The focus of work presented in this thesis from *Chapter 3* onwards is on the class IA PI3K isoform of p110δ. Mice that lack enzymatically active p110δ, $p110\delta^{D910A/D910A}$ mice, develop colitis [63]. p110δ is also critical in mast cell degranulation [64]. Additionally, high levels of p110δ can be found in several cancers of non-leukocyte origin [65]. This makes p110δ an attractive therapeutic target and p110δ-specific inhibitors are currently being developed.

Despite the importance of the p110δ PI3K subunit, its underlying biology, especially the regulation of the tissue-specific expression of p110δ, remains poorly understood. The aim of the work presented in *Chapter 3* of this thesis was to increase the understanding of the regulation of expression of p110δ PI3K. Expression of *PIK3CD*, the p110δ gene, is complex, with multiple transcription start sites, indicative of the presence of multiple distinct promoters which are mainly used in leukocytes. For the first time a conserved promoter region of *PIK3CD*, is identified that is highly active in leukocytes and accounts for the predominantly leukocyte-restricted expression of p110δ.

**Action of p110δ PI3K**

The complex regulation of p110δ raises questions regarding the function of this enzyme in cellular physiology. However, studying the function of any enzyme without a priori assumptions as to the pathways targeted is highly complicated. In order to address this issue, in *Chapter 4* the use of peptide arrays for studying cellular biochemistry was explored. The usefulness of peptide arrays for studying signal transduction was demonstrated by the generation of the first comprehensive description of the kinetics of phosphorylation events induced by lipopolysaccharide (LPS) stimulation. Further confidence in the usefulness of peptide array technology came from Western blot analysis, which corroborated the signals obtained using peptide arrays.

$p110\delta^{D910A/D910A}$ mice develop inflammatory bowel disease (IBD) [63]. IBD is thought to arise as a result of abnormal innate immunity to enteric flora [66]. Various

studies link PI3Ks to the innate immune system. p85$\alpha^{-/-}$ mice develop increased immune responses upon *Leishmania major* infections [67] and pharmacological inhibition of PI3K activity exacerbates microbial sepsis [68,69]. Furthermore, microbial agents that activate cells of the immune system to produce cytokines also activate class IA PI3Ks [67,70-74] and PI3Ks suppress cytokine production triggered as was shown by an increase in cytokine production upon absence or inhibition of PI3K [67,73,75] In *Chapter 5* the high throughput analysis of signal transduction described in *Chapter 4* was used to study the role of p110δ PI3K in innate immune signalling. Macrophages genetically deficient in p110δ enzymatic activity (p110$\delta^{D910A/D910A}$) were compared to wildtype macrophages, revealing a modulatory role of p110δ in LPS dependent signalling.

**Function of p110δ PI3K**

The final challenge remaining is relating expression and action of p110δ PI3K to its actual function in pathophysiology. As the p110$\delta^{D910A/D910A}$ mice develop spontaneous colitis, and both PI3Ks and colitis are linked to cancer, the function of this enzyme has been studied in a colitis associated cancer model, described in *Chapter 6*. Here we observe a surprising uncoupling of the development of cancer and the colitis itself in the absence of active p110δ. In *Chapter 7* the work presented in this thesis is summarised and discussed.

In summary, this thesis provides insight into the expression, action and function of p110δ PI3K.

## References


[1] Vanhaesebroeck, B. et al. (2001). Synthesis and function of 3-phosphorylated inositol lipids. Annu Rev Biochem 70, 535-602.

[2] Vanhaesebroeck, B. and Waterfield, M.D. (1999). Signaling by distinct classes of phosphoinositide 3-kinases. Exp Cell Res 253, 239-54.

[3] Nock, G. (2008). Investigating the isoform-specific role of phosphoinositide 3-kinase p110delta. Thesis Submitted in fulfilment of the requirements for the degree of Doctor of Philosophy from University College London

[4] Rameh, L.E. and Cantley, L.C. (1999). The Role of Phosphoinositide 3-Kinase Lipid Products in Cell Function. J. Biol. Chem. 274, 8347-8350.

[5] Vanhaesebroeck, B., Leevers, S.J., Panayotou, G. and Waterfield, M.D. (1997). Phosphoinositide 3-kinases: a conserved family of signal transducers. Trends Biochem Sci 22, 267-72.

[6] Sotsios, Y. and Ward, S.G. (2000). Phosphoinositide 3-kinase: a key biochemical signal for cell migration in response to chemokines. Immunol Rev 177, 217-35.

[7] Vanhaesebroeck, B. et al. (1999). Distinct PI(3)Ks mediate mitogenic signalling and cell migration in macrophages. Nat Cell Biol 1, 69-71.

[8] Lemmon, M.A. and Ferguson, K.M. (2000). Signal-dependent membrane targeting by pleckstrin homology (PH) domains. Biochem J 350 Pt 1, 1-18.

[9] Lindmo, K. and Stenmark, H. (2006). Regulation of membrane traffic by phosphoinositide 3-kinases. J Cell Sci 119, 605-14.

[10] Stenmark, H. and Aasland, R. (1999). FYVE-finger proteins--effectors of an inositol lipid. J Cell Sci 112 ( Pt 23), 4175-83.

[11] Lemmon, M.A. and Ferguson, K.M. (2001). Molecular determinants in pleckstrin homology domains that allow specific recognition of phosphoinositides. Biochem. Soc. Trans. 29, 377-384.

[12] Rodriguez-Viciana, P., Warne, P.H., Dhand, R., Vanhaesebroeck, B., Gout, I., Fry, M.J., Waterfield, M.D. and Downward, J. (1994). Phosphatidylinositol-3-OH kinase as a direct target of Ras. Nature 370, 527-32.

[13] Voigt, P., Brock, C., Nurnberg, B. and Schaefer, M. (2005). Assigning functional domains within the p101 regulatory subunit of phosphoinositide 3-kinase gamma. J Biol Chem 280, 5121-7.

[14] Hiles, I.D. et al. (1992). Phosphatidylinositol 3-kinase: structure and expression of the 110 kd catalytic subunit. Cell 70, 419-29.

[15] Hu, P., Mondino, A., Skolnik, E.Y. and Schlessinger, J. (1993). Cloning of a novel, ubiquitously expressed human phosphatidylinositol 3-kinase and identification of its binding site on p85. Mol Cell Biol 13, 7677-88.

[16] Vanhaesebroeck, B. et al. (1997). p110delta , a novel phosphoinositide 3-kinase in leukocytes. PNAS 94, 4330-4335.

[17] Escobedo, J.A., Navankasattusas, S., Kavanaugh, W.M., Milfay, D., Fried, V.A. and Williams, L.T. (1991). cDNA cloning of a novel 85 kd protein that has SH2 domains and regulates binding of PI3-kinase to the PDGF beta-receptor. Cell 65, 75-82.

[18] Otsu, M. et al. (1991). Characterization of two 85 kd proteins that associate with receptor tyrosine kinases, middle-T/pp60c-src complexes, and PI3-kinase. Cell 65, 91-104.

[19] Skolnik, E.Y., Margolis, B., Mohammadi, M., Lowenstein, E., Fischer, R., Drepps, A., Ullrich, A. and Schlessinger, J. (1991). Cloning of PI3 kinase-associated p85 utilizing a novel method for expression/cloning of target proteins for receptor tyrosine kinases. Cell 65, 83-90.

[20] Antonetti, D.A., Algenstaedt, P. and Kahn, C.R. (1996). Insulin receptor substrate 1 binds two novel splice variants of the regulatory subunit of phosphatidylinositol 3-kinase in muscle and brain. Mol. Cell. Biol. 16, 2195-2203.

[21] Fruman, D.A., Cantley, L.C. and Carpenter, C.L. (1996). Structural organization and alternative splicing of the murine phosphoinositide 3-kinase p85 alpha gene. Genomics 37, 113-21.

[22] Inukai, K. et al. (1996). A novel 55-kDa regulatory subunit for phosphatidylinositol 3-kinase structurally similar to p55PIK Is generated by alternative splicing of the p85alpha gene. J Biol Chem 271, 5317-20.

[23] Inukai, K. et al. (1997). p85alpha gene generates three isoforms of regulatory subunit for phosphatidylinositol 3-kinase (PI 3-Kinase), p50alpha, p55alpha, and p85alpha, with different PI 3-kinase activity elevating responses to insulin. J Biol Chem 272, 7873-82.

[24] Pons, S. et al. (1995). The structure and function of p55PIK reveal a new regulatory subunit for phosphatidylinositol 3-kinase. Mol Cell Biol 15, 4453-65.
[25] Bi, L., Okabe, I., Bernard, D.J., Wynshaw-Boris, A. and Nussbaum, R.L. (1999). Proliferative defect and embryonic lethality in mice homozygous for a deletion in the p110alpha subunit of phosphoinositide 3-kinase. J Biol Chem 274, 10963-8.
[26] Foukas, L.C. et al. (2006). Critical role for the p110alpha phosphoinositide-3-OH kinase in growth and metabolic regulation. Nature 441, 366-70.
[27] Bi, L., Okabe, I., Bernard, D.J. and Nussbaum, R.L. (2002). Early embryonic lethality in mice deficient in the p110beta catalytic subunit of PI 3-kinase. Mamm Genome 13, 169-72.
[28] Yu, J., Zhang, Y., McIlroy, J., Rordorf-Nikolic, T., Orr, G.A. and Backer, J.M. (1998). Regulation of the p85/p110 phosphatidylinositol 3'-kinase: stabilization and inhibition of the p110alpha catalytic subunit by the p85 regulatory subunit. Mol Cell Biol 18, 1379-87.
[29] Hawkins, P.T., Anderson, K.E., Davidson, K. and Stephens, L.R. (2006). Signalling through Class I PI3Ks in mammalian cells. Biochem Soc Trans 34, 647-62.
[30] Stoyanov, B. et al. (1995). Cloning and characterization of a G protein-activated human phosphoinositide-3 kinase. Science 269, 690-3.
[31] Stephens, L.R. et al. (1997). The G beta gamma sensitivity of a PI3K is dependent upon a tightly associated adaptor, p101. Cell 89, 105-14.
[32] Voigt, P., Dorner, M.B. and Schaefer, M. (2006). Characterization of p87PIKAP, a novel regulatory subunit of phosphoinositide 3-kinase gamma that is highly expressed in heart and interacts with PDE3B. J Biol Chem 281, 9977-86.
[33] Suire, S., Coadwell, J., Ferguson, G.J., Davidson, K., Hawkins, P. and Stephens, L. (2005). p84, a New G[beta][gamma]-Activated Regulatory Subunit of the Type IB Phosphoinositide 3-Kinase p110[gamma]. Current Biology 15, 566-570.
[34] Krugmann, S., Hawkins, P.T., Pryer, N. and Braselmann, S. (1999). Characterizing the Interactions between the Two Subunits of the p101/p110gamma Phosphoinositide 3-Kinase and Their Role in the Activation of This Enzyme by Gbeta gamma Subunits. J. Biol. Chem. 274, 17152-17158.
[35] Brown, R.A., Domin, J., Arcaro, A., Waterfield, M.D. and Shepherd, P.R. (1999). Insulin activates the alpha isoform of class II phosphoinositide 3-kinase. J Biol Chem 274, 14529-32.
[36] Brown, R.A. and Shepherd, P.R. (2001). Growth factor regulation of the novel class II phosphoinositide 3-kinases. Biochem Soc Trans 29, 535-7.
[37] Soos, M.A., Jensen, J., Brown, R.A., O'Rahilly, S., Shepherd, P.R. and Whitehead, J.P. (2001). Class II phosphoinositide 3-kinase is activated by insulin but not by contraction in skeletal muscle. Arch Biochem Biophys 396, 244-8.
[38] Arcaro, A., Zvelebil, M.J., Wallasch, C., Ullrich, A., Waterfield, M.D. and Domin, J. (2000). Class II phosphoinositide 3-kinases are downstream targets of activated polypeptide growth factor receptors. Mol Cell Biol 20, 3817-30.
[39] Ktori, C., Shepherd, P.R. and O'Rourke, L. (2003). TNF-alpha and leptin activate the alpha-isoform of class II phosphoinositide 3-kinase. Biochem Biophys Res Commun 306, 139-43.
[40] Maffucci, T., Cooke, F.T., Foster, F.M., Traer, C.J., Fry, M.J. and Falasca, M. (2005). Class II phosphoinositide 3-kinase defines a novel signaling pathway in cell migration. J Cell Biol 169, 789-99.
[41] Turner, S.J., Domin, J., Waterfield, M.D., Ward, S.G. and Westwick, J. (1998). The CC chemokine monocyte chemotactic peptide-1 activates both the class I p85/p110 phosphatidylinositol 3-kinase and the class II PI3K-C2alpha. J Biol Chem 273, 25987-95.
[42] Zhang, J., Banfic, H., Straforini, F., Tosi, L., Volinia, S. and Rittenhouse, S.E. (1998). A type II phosphoinositide 3-kinase is stimulated via activated integrin in platelets. A source of phosphatidylinositol 3-phosphate. J Biol Chem 273, 14081-4.
[43] Wheeler, M. and Domin, J. (2001). Recruitment of the class II phosphoinositide 3-kinase C2beta to the epidermal growth factor receptor: role of Grb2. Mol Cell Biol 21, 6660-7.
[44] Gaidarov, I., Smith, M.E., Domin, J. and Keen, J.H. (2001). The class II phosphoinositide 3-kinase C2alpha is activated by clathrin and regulates clathrin-mediated membrane trafficking. Mol Cell 7, 443-9.
[45] Wheeler, M. and Domin, J. (2006). The N-terminus of phosphoinositide 3-kinase-C2beta regulates lipid kinase activity and binding to clathrin. J Cell Physiol 206, 586-93.
[46] Falasca, M. and Maffucci, T. (2007). Role of class II phosphoinositide 3-kinase in cell signalling. Biochem Soc Trans 35, 211-4.
[47] Traer, C.J., Foster, F.M., Abraham, S.M. and Fry, M.J. (2006). Are class II phosphoinositide 3-kinases potential targets for anticancer therapies? Bull Cancer 93, E53-8.

[48] Backer, J.M. (2008). The regulation and function of Class III PI3Ks: novel roles for Vps34. Biochem J 410, 1-17.
[49] Muraille, E., Pesesse, X., Kuntz, C. and Erneux, C. (1999). Distribution of the src-homology-2-domain-containing inositol 5-phosphatase SHIP-2 in both non-haemopoietic and haemopoietic cells and possible involvement of SHIP-2 in negative signalling of B-cells. Biochem J 342 Pt 3, 697-705.
[50] Cully, M., You, H., Levine, A.J. and Mak, T.W. (2006). Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. Nat Rev Cancer 6, 184-92.
[51] Harris, S.J., Parry, R.V., Westwick, J. and Ward, S.G. (2008). Phosphoinositide lipid phosphatases: natural regulators of phosphoinositide 3-kinase signaling in T lymphocytes. J Biol Chem 283, 2465-9.
[52] Rao, A. (1994). NF-ATp: a transcription factor required for the co-ordinate induction of several cytokine genes. Immunol Today 15, 274-81.
[53] Rohrschneider, L.R., Fuller, J.F., Wolf, I., Liu, Y. and Lucas, D.M. (2000). Structure, function, and biology of SHIP proteins. Genes Dev 14, 505-20.
[54] Zhang, S.J. et al. (2006). The investigation of mutation and single nucleotide polymorphism of receptor tyrosine kinases and downstream scaffold molecules in acute myeloid leukemia. Leuk Lymphoma 47, 2610-6.
[55] Luo, J.M., Liu, Z.L., Hao, H.L., Wang, F.X., Dong, Z.R. and Ohno, R. (2004). Mutation analysis of SHIP gene in acute leukemia. Zhongguo Shi Yan Xue Ye Xue Za Zhi 12, 420-6.
[56] Berridge, M.J. (1987). Inositol trisphosphate and diacylglycerol: two interacting second messengers. Annu Rev Biochem 56, 159-93.
[57] Coffer, P.J., Jin, J. and Woodgett, J.R. (1998). Protein kinase B (c-Akt): a multifunctional mediator of phosphatidylinositol 3-kinase activation. Biochem J 335 ( Pt 1), 1-13.
[58] Brazil, D.P., Yang, Z.-Z. and Hemmings, B.A. (2004). Advances in protein kinase B signalling: AKTion on multiple fronts. Trends in Biochemical Sciences 29, 233-242.
[59] Vanhaesebroeck, B. and Alessi, D.R. (2000). The PI3K-PDK1 connection: more than just a road to PKB. Biochem J 346 Pt 3, 561-76.
[60] Alessi, D.R., Andjelkovic, M., Caudwell, B., Cron, P., Morrice, N., Cohen, P. and Hemmings, B.A. (1996). Mechanism of activation of protein kinase B by insulin and IGF-1. EMBO J 15, 6541-51.
[61] Alessi, D.R., James, S.R., Downes, C.P., Holmes, A.B., Gaffney, P.R., Reese, C.B. and Cohen, P. (1997). Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Balpha. Curr Biol 7, 261-9.
[62] Sarbassov, D.D., Guertin, D.A., Ali, S.M. and Sabatini, D.M. (2005). Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 307, 1098-101.
[63] Okkenhaug, K. et al. (2002). Impaired B and T cell antigen receptor signaling in p110delta PI 3-kinase mutant mice. Science 297, 1031-4.
[64] Ali, K. et al. (2004). Essential role for the p110delta phosphoinositide 3-kinase in the allergic response. Nature 431, 1007-11.
[65] Sawyer, C., Sturge, J., Bennett, D.C., O'Hare, M.J., Allen, W.E., Bain, J., Jones, G.E. and Vanhaesebroeck, B. (2003). Regulation of breast cancer cell chemotaxis by the phosphoinositide 3-kinase p110delta. Cancer Res 63, 1667-75.
[66] Hisamatsu, T., Ogata, H. and Hibi, T. (2008). Innate immunity in inflammatory bowel disease: state of the art. Curr Opin Gastroenterol 24, 448-54.
[67] Fukao, T. et al. (2002). PI3K-mediated negative feedback regulation of IL-12 production in DCs. Nat Immunol 3, 875-81.
[68] Williams, D.L. et al. (2004). Modulation of the phosphoinositide 3-kinase pathway alters innate resistance to polymicrobial sepsis. J Immunol 172, 449-56.
[69] Schabbauer, G., Tencati, M., Pedersen, B., Pawlinski, R. and Mackman, N. (2004). PI3K-Akt pathway suppresses coagulation and inflammation in endotoxemic mice. Arterioscler Thromb Vasc Biol 24, 1963-9.
[70] Arbibe, L. et al. (2000). Toll-like receptor 2-mediated NF-kappa B activation requires a Rac1-dependent pathway. Nat Immunol 1, 533-40.
[71] Ardeshna, K.M., Pizzey, A.R., Devereux, S. and Khwaja, A. (2000). The PI3 kinase, p38 SAP kinase, and NF-kappaB signal transduction pathways are involved in the survival and maturation of lipopolysaccharide-stimulated human monocyte-derived dendritic cells. Blood 96, 1039-46.
[72] Fukao, T. and Koyasu, S. (2003). PI3K and negative regulation of TLR signaling. Trends Immunol 24, 358-63.

[73] Guha, M. and Mackman, N. (2002). The phosphatidylinositol 3-kinase-Akt pathway limits lipopolysaccharide activation of signaling pathways and expression of inflammatory mediators in human monocytic cells. J Biol Chem 277, 32124-32.
[74] Koyasu, S. (2003). The role of PI3K in immune cells. Nat Immunol 4, 313-9.
[75] Diaz-Guerra, M.J., Castrillo, A., Martin-Sanz, P. and Bosca, L. (1999). Negative regulation by phosphatidylinositol 3-kinase of inducible nitric oxide synthase expression in macrophages. J Immunol 162, 6184-90.